



Taxonomic reexamination of *Portulaca boninensis* (Portulacaceae) in the Bonin (Ogasawara) Islands of Japan using molecular and morphological data

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Abstract

Molecular phylogenetic analyses, morphological observations, and nomenclatural studies were carried out to investigate the taxonomic status of *Portulaca boninensis*, endemic species from the Bonin (Ogasawara) Islands (Japan). The results addressed controversy between the widely naturalized *P. boninensis* and *P. pilosa*, indicating that they are phylogenetically and morphologically distinct. Furthermore, *P. boninensis* was showed to be conspecific to *P. psammotropha* which is until now recorded in southern China, Taiwan, and the northern Philippines, but not in the Ryukyus. The name of *P. psammotropha* was lectotypified on a specimen preserved at K. Based on phylogeography, *P. psammotropha* likely migrated to the Oceanic Bonin Islands oversea by sea-current dispersal or by exo- and/or end oozochory through migratory birds without passing through Ryukyus.

Key words: Biogeography, ITS, lectotype, Philippines, *Portulaca psammotropha*, Taiwan, Taxonomic revision

Introduction

Portulaca Linnaeus (1753: 445) is the only genus belongs to Portulacaceae Juss. a monotypic family according to the molecular studies by Nyffeler & Egli (2010), and Ocampo & Columbus (2012). The genus has a worldwide distribution, mostly in the tropics and subtropics, and comprises over than 100 species (Ocampo & Columbus 2012). *P. boninensis* Tuyama (1939: 6) was described on the basis of a specimen collected from Chichi-jima Island of the Bonin (Ogasawara) Islands, which are oceanic islands about 1000 km south of Japan proper. Previously, this species has been recorded in five of the Bonin Islands, namely, Nakohdo-jima, Chichi-jima, Muko-jima, Iwo-to (Kobayashi & Ono 1987), and Minami-iwo-to (Fujita *et al.* 2008) (Fig. 1). Populations of *P. boninensis* in Muko-jima Island have disappeared, and they rarely occur in the other four islands (Kato H., pers. comm.). Because of its narrow distribution range and rarity in Japan, *P. boninensis* is considered a threatened species at metropolitan (Kato & Ohba 2014) and regional levels (Ono *et al.* 1986).

The taxonomic treatment of *P. boninensis* as a Japanese endemic species (Tuyama 1939) has been accepted in subsequent studies (Ono *et al.* 1986, Kobayashi & Ono 1987, Kato & Ohba 2014). However, Kato & Ohba (2014) mentioned that *P. boninensis* has been often misunderstood as *P. pilosa* Linnaeus (1753: 445), which is native to South America, and is now widely naturalized in the tropics and subtropics (PIER 2013) including the Bonin Islands (Kobayashi & Ono 1987). Because of the taxonomic complication, Toyoda (2003) considered the Bonin plants as an alien plant. Therefore, Geesink (1969), Fujita *et al.* (2008) and Toyoda (2014) discussed the need for taxonomic reexamination of *P. boninensis*.

In this report, we explain the taxonomic confusion about *P. boninensis* by elucidating phylogenetic relationships between *P. boninensis* and other morphologically similar taxa occurring in Japan and Taiwan, including *P. pilosa*, and by clarifying morphological differences.

TABLE 1. *Portulaca* taxa investigated and their voucher specimens, collection areas and DDBJ accession number of ITS sequences. Asterisks explanations: *) taxonomic treatment following Tuyama (1938); **) data from Ocampo & Columbus (2012); ^b) from Kokubugata *et al.* (2013); ^c) from the present study.

Taxon	Collection number (Herbarium)	Collection area	DDBJ accession number**
INGROUP			
<i>Portulaca amilis</i> Speng.	Ocampo <i>et al.</i> 1556 (RSA, SI)	Argentina	JF508527 ^a
<i>P. australis</i> Endl.	Ocampo <i>et al.</i> 1747 (BRI, RSA)	Australia	JF508531 ^a
<i>P. bicolor</i> F.Muell.	Ocampo <i>et al.</i> 1753 (BRI, RSA)	Australia	JF508532 ^a
<i>P. boninensis</i> Tuyama*	<i>G.Kokubugata</i> 17300(TNS)	Japan, Bonin, Chichi-jima Isl.	LC018836 ^c
<i>P. boninensis</i> Tuyama*	<i>G.Kokubugata</i> 17301(TNS)	Japan, Bonin, Chichi-jima Isl.	LC018837 ^c
<i>P. boninensis</i> Tuyama*	<i>G.Kokubugata</i> 17302(TNS)	Japan, Bonin, Chichi-jima Isl.	LC018838 ^c
<i>P. boninensis</i> Tuyama*	<i>H.Kato & K.Shimada</i> K0106(MAK)	Japan, Bonin, Minami-io-to Isl.	LC018840 ^c
<i>P. boninensis</i> Tuyama*	<i>H.Kato & K.Shimada</i> K0107(MAK)	Japan, Bonin, Minami-io-to Isl.	LC018841 ^c
<i>P. californica</i> D.Legrand	Ocampo & Columbus 1529 (RSA)	Mexico	JF508534 ^a
<i>P. confertifolia</i> Hauman	Ocampo <i>et al.</i> 1619 (RSA, SI)	Argentina	JF508536 ^a
<i>P. decipiens</i> Poelln.	Ocampo <i>et al.</i> 1758 (BRI, RSA)	Australia	JF508539 ^a
<i>P. eruca</i> Hauman	Ocampo <i>et al.</i> 1645 (RSA, SI)	Argentina	JF508543 ^a
<i>P. filifolia</i> F.Muell.	Ocampo <i>et al.</i> 1733 (BRI, RSA)	Australia	JF508544 ^a
<i>P. foliosa</i> Ker Gawl.	Ocampo 1772cv (RSA)	Tropical Africa (cultivated)	JF508546 ^a
<i>P. giliesii</i> Hook.	Ocampo <i>et al.</i> 1545 (RSA, SI)	Argentina	JF508548 ^a
<i>P. grandiflora</i> Hook.	Ocampo <i>et al.</i> 1662 (RSA, SI)	Argentina	JF508549 ^a
<i>P. grandiflora</i> Hook. cv.	Ocampo 1403cv (RSA)	Cultivated	JF508550 ^a
<i>P. halimoides</i> L.	Ocampo 1474 (RSA)	Mexico	JF508552 ^a
<i>P. massaica</i> S.M.Phillips	Cruse-Sanders <i>s.n.</i> (RSA)	Tanzania	JF508559 ^a
<i>P. matthewsii</i> G.Ocampo	Ocampo 1425 (RSA)	Mexico	JF508560 ^a
<i>P. mexicana</i> P.Wilson	Ocampo & Morales 1461 (RSA)	Mexico	JF508561 ^a
<i>P. mucronulata</i> D.Legrand	Ocampo <i>et al.</i> 1598 (RSA, SI)	Argentina	JF508563 ^a
<i>P. obtusa</i> Poelln.	Ocampo <i>et al.</i> 1591 (RSA, SI)	Argentina	JF508565 ^a
<i>P. okinawensis</i> Walker et Tawada	<i>G.Kokubugata</i> 12873 (TNS)	Japan, Ryukyus, Okinawa Isl.	AB823833 ^b
var. <i>okinawensis</i>			
<i>P. okinawensis</i> Walker et Tawada	<i>G.Kokubugata</i> 13068 (TNS)	Japan, Ryukyus, Tonaki Isl.	AB823845 ^b
var. <i>okinawensis</i>			
<i>P. okinawensis</i> var. <i>amamiensis</i>	<i>G.Kokubugata</i> 15198 (TNS)	Japan, Ryukyus, Amami Isl.	AB823828 ^b
Kokubugata <i>et al.</i>			
<i>P. okinawensis</i> var. <i>amamiensis</i>	<i>G.Kokubugata</i> 12141 (TNS)	Japan, Ryukyus, Tokuno-shima Isl.	AB823831 ⁿ
Kokubugata <i>et al.</i>			
<i>P. papulifera</i> D.Legrand	Ocampo <i>et al.</i> 1569 (RSA, SI)	Argentina	JF508580 ^a
<i>P. perennis</i> R.E.Fr.	Ocampo <i>et al.</i> 1606 (RSA, SI)	Argentina	JF508581 ^a
<i>P. pilosa</i> L.	Nortrup <i>s.n.</i> (UNCC)	USA	JF508585 ^a
<i>P. pilosa</i> L.	<i>G.Kokubugata</i> 17303(TNS)	Japan, Bonin, Chichi-jima Isl.	LC018839 ^c
<i>P. psammotropha</i> Hance	<i>G.Kokubugata</i> 13338 (TNS)	Taiwan, Hsiao-liuqie Isl.	AB823850 ^b
<i>P. pusilla</i> Kunth	Gröger 927 (MO)	Venezuela	JF508587 ^a
<i>P. rubricaulis</i> Kunth	Simá <i>et al.</i> 2433 (MO)	Mexico	JF508592 ^a
<i>P. rzedowskiana</i> G.Ocampo	Ocampo 1124 (IEB)	Mexico	JF508593 ^a
<i>P. sclerocarpa</i> A.Gray	Morden 1828 (HAW)	Hawaii	JF508594 ^a
<i>P. smallii</i> P.Wilson	Herkenham <i>s.n.</i> (UNCC)	USA	JF508595 ^a
<i>P. suffrutescens</i> Engelm.	Ocampo & Columbus 1505 (RSA)	Mexico	JF508597 ^a
<i>P. tingoensis</i> J.F.Macbr.	Ocampo <i>et al.</i> 1615 (RSA, SI)	Argentina	JF508598 ^a
<i>P. tuberosa</i> Roxb.	Ocampo <i>et al.</i> 1737 (BRI, RSA)	Australia	JF508599 ^a
<i>P. villosa</i> Cham.	Perlman 13305 (PTBG)	Hawaii	JF508604 ^a
OUTGROUP			
<i>P. elatior</i> Mart. ex Rohrb.	Ocampo 1708cv (RSA)	Caribbean (cultivated)	JF508542

Materials and Methods

Morphology

Four *Portulaca* plants were collected from one locality in Chichi-jima Island, two plants from one locality of Minami-iwo-to Island (Table 1; Fig. 1). On the basis of Tuyama (1939), and Takahashi (2003), the plants with yellow petals were treated as *P. boninensis*, while those with reddish-violet petals as *P. pilosa*. Specimens examined were deposited in the herbaria MAK and TNS (acronyms according to Thiers 2011) (Table 1).

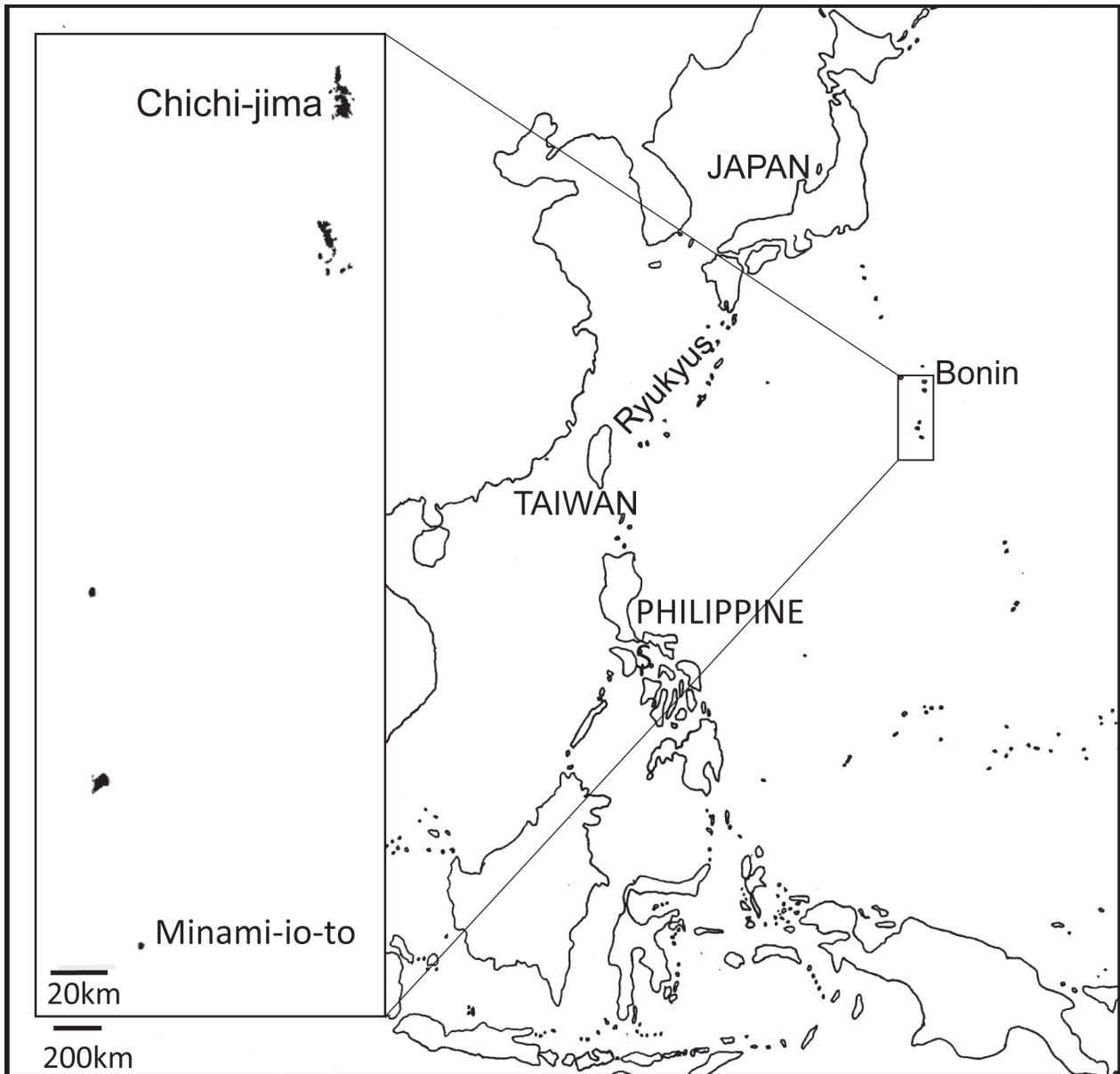


FIGURE 1. Geographic location of Chichi-jima and Minami-iwo-to islands (the Bonin Islands) where *Portulaca boninensis* was collected.

DNA extraction, polymerase chain reaction, and sequencing

Total DNA was extracted from dried leaves using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols. The total DNA samples isolated were deposited in the Molecular Biodiversity Research Center of the National Museum of Nature and Science, Japan. Voucher specimens for the present molecular analyses were deposited in the herbarium of Academia Sinica, Taipei (HAST), MAK, and TNS.

The entire Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA, including ITS1, 5.8S, and ITS2, was amplified by polymerase chain reaction (PCR) on an iCycler (Bio-Rad, Hercules, CA, USA). The forward primer AB101 (5'-ACG AAT TCA AGG TCC GGT GAA GTG TTC G-3') and reverse primer AB102 (5'-TAG AAT TCC CCG GTT CGC TCG CCG TTA C-3') (Douzery *et al.* 1999) were used for PCR amplification. Amplifications were performed using EmeraldAmp PCR Master Mix dye (Takara, Otsu, Japan). The PCR profile comprised 35 cycles of 1 min at 94°C, 30 s at 55°C, and 1.5 min at 72°C, after an initial denaturing for 3 min at 94°C. PCR products were checked by electrophoresis before purification with the ExoStar clean-up kit (USB, Cleveland, OH, USA). Cycle sequencing was performed with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA, USA) using PCR primers listed above with an additional internal reverse primer N2 (5'-GGC GCA ACT TGC GTT CAA-3') and the forward primer N3 (5'-GCT CTC GCA GCA TCG ATG AAG-3') (Yukawa T, TNS, personal communication). Cycle sequencing products were then purified by ethanol precipitation. Automated sequencing was performed with an Applied Biosystems 3130xl Genetic Analyzer. The electropherograms were assembled using the ATGC ver. 4.01 software (Genetyx Co., Tokyo, Japan). Sequence data from this study were deposited in the DNA Data Bank of Japan (DDBJ) database (<http://www.ddbj.nig.ac.jp/>).

ITS data from DNA database

To elucidate the phylogenetic relationship between *P. boninensis* and related species, ITS data previously provided by Ocampo & Columbus (2012), and Kokubugata *et al.* (2013) were considered. A total of 36 accessions for 34 *Portulaca* taxa were obtained from DDBJ (Table 1). Our previous study found two ITS types in each of the two varieties of *P. okinawensis* var. *okinawensis* Walker et Tawada (1951: 138) and var. *amamiensis* Kokubugata, Koh Nakam. & Yokota (2013: 21), and one ITS type in seven plants of *P. psammotropha* Hance (1851: 660) from four localities in southern Taiwan (Kokubugata *et al.* 2013). We used each ITS type of *P. okinawensis* and *P. psammotropha* as an operational taxonomic unit (OTU) in the analyses. For the outgroup, we referred to Kokubugata *et al.* (2013) and considered *P. elatior* Martius ex Rohrbach (1872: 302).

Phylogenetic analyses

DNA sequences were aligned using the ClustalW 1.8 software (Thompson *et al.* 1994) and then manually adjusted. Phylogenetic analyses were conducted based on a Bayesian approach using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and a maximum parsimony (MP) criterion using PAUP* version 4.0b10 (Swofford 2002).

In the Bayesian phylogenetic analysis, MrModeltest 2.2 (Nylander 2004) was used to estimate the appropriate evolutionary model of nucleotide substitutions. Based on the model selected, two separate runs of Metropolis coupled Markov chain Monte Carlo (MCMCMC) analyses were performed, each with a random starting tree and four chains (one cold and three heated). The MCMCMC length was one million generations and the chain was sampled every one-hundredth generation from the cold chain. The mixing and convergence of the MCMCMC chains of the two runs was assessed by inspection of the trace plots of parameters using TRACER version 1.5.0 (Drummond & Rambaut 2007); the first 2,500 sample trees (25% of the total 10,000 sample trees) were discarded as burn-in. After the burn-in, the effective sample sizes (ESS) of all parameters were > 200, indicating that the analyses sampled the posterior distributions of each parameter satisfactorily, and the average standard deviation of split frequencies (ASDSF) were < 0.01. The potential scale reduction factors (PSRFs) were ascertained to be reasonably close to 1.0 for all parameters in an output table. A 50% majority consensus tree of the output tree file from MrBayes was generated using TREEVIEW (Page 1996).

In the MP phylogenetic analysis, indels were treated as missing data. Characters were treated as unordered, and character transformations were weighted equally. The branch collapse option was set to collapse at a minimum length of zero. A heuristic parsimony search was performed with 200 replicates of random additions of sequences with ACCTRAN character optimization, tree bisection-reconnection (TBR) branch swapping, and MULTREES and STEEPEST DESCENT options on. Statistical support for each clade was assessed using bootstrap analysis (Felsenstein 1985). Ten thousand replicates of heuristic searches, with the TBR branch swapping switched on and MULTREES options off, were performed to calculate bootstrap values.

Results

Morphology

Three out of the four plants from Chichi-jima Island (*G. Kokubugata* 17300, 17301 & 17302; Table 1) and the two plants from Minami-iwo-to Island (*H. Kato* & *K. Shimada* K0106 & K0107) had yellow petals characteristics, resembling *P. boninensis* (Fig. 2A & B). The other plant from Chichi-jima Island (*G. Kokubugata* 17303 in Table 1) had reddish violet petals characteristic, resembling *P. pilosa* (Fig. 2F). All Bonin samples, both *P. boninensis* and *P. pilosa*, had axillary hairs (arrows in Fig. 2A & F).

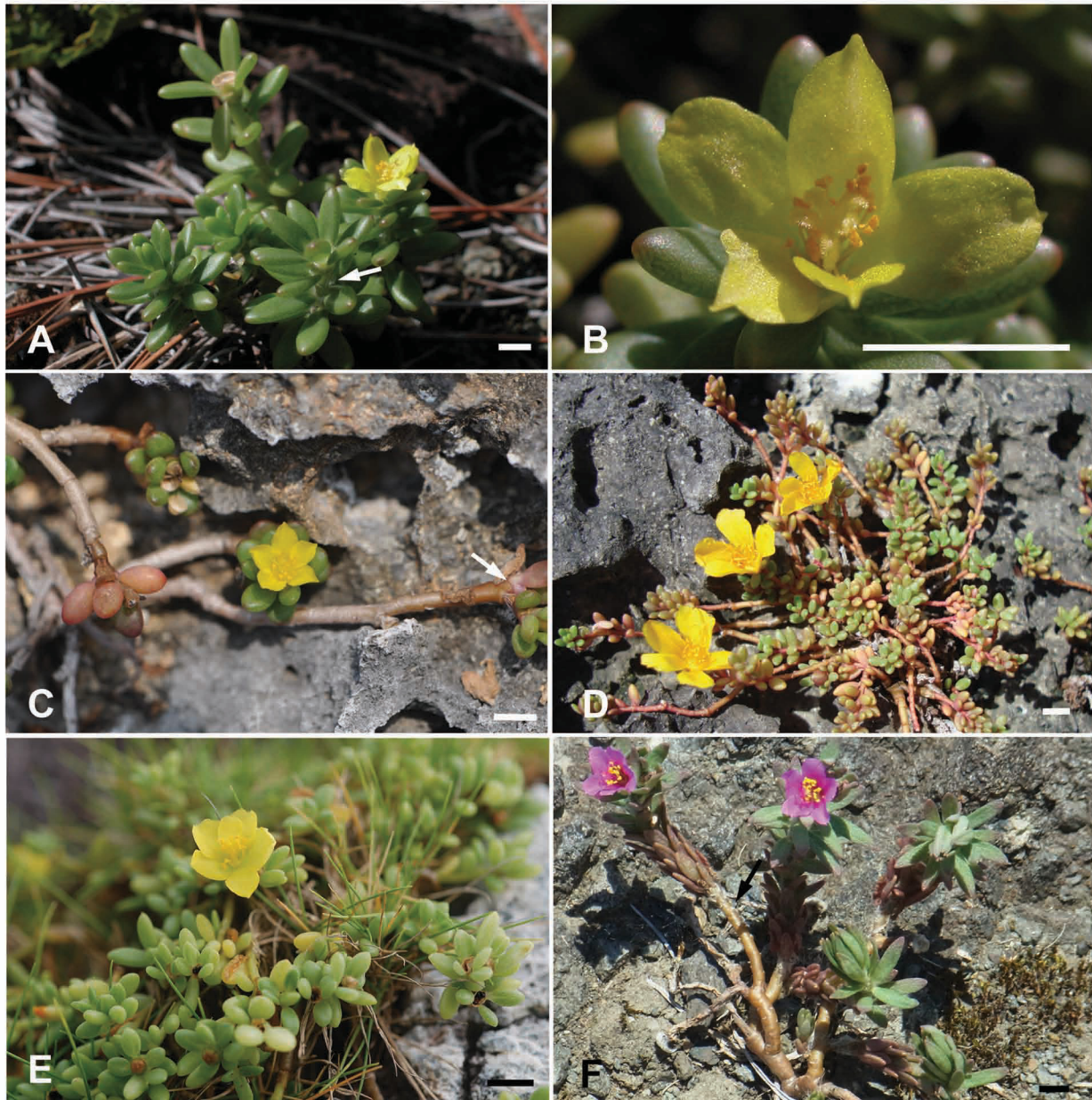


FIGURE 2. *Portulaca boninensis* and related species. **A–B)** Habitat and flower of *P. boninensis* (*G. Kokubugata*17300-17302, Chichi-jima Island of the Bonin Islands, Japan). **C)** *P. psammotropha* (*G. Kokubugata*13338, Hsiao-liuqie Island, Taiwan). **D)** *P. okinawensis* var. *okinawensis* (*G. Kokubugata*13338, Okinawa Island of the Ryukyus, Japan). **E)** *P. okinawensis* var. *amamiensis* (*G. Kokubugata*15198, Amami Island of the Ryukyus, Japan). **F)** *P. pilosa* (*G. Kokubugata*17303, Chichi-jima Island of the Bonin Islands, Japan). Scale bars, 5 mm. Arrows in A, C, and F indicate axillary hairs.

Phylogenetic relationships based on ITS

ITS sequences were identical among five plants identified as *P. boninensis* (three and two from Chichi-jima and Mimami-io-to islands, respectively). So, we used the identical ITS type as an OTU in the Bayesian and MP phylogenetic analyses. ITS sequence of the plant identified as *P. pilosa* from Chichi-jima Island was different from that of *P. pilosa* collected in the USA (Ocampo & Columbus 2012), and both were used as OTUs. After alignment of the 38 OTUs of 35 taxa (including outgroup), we obtained a matrix of 628 bp.

In the Bayesian analysis, the model of GTR+G was selected. The 50% majority rule consensus tree of all the post-burn-in trees was depicted with Bayesian posterior probabilities (PPs) (see Fig. 3). In the MP analysis, 97 out of 179 variable characters were parsimony informative, and 474 equally most parsimonious trees of 261 steps were obtained with a consistency index (CI) of 0.801, a retention index (RI) of 0.882, and a rescaled consistency index (RC) of 0.706. The topology of the strict consensus tree was highly compatible with that of the Bayesian tree, except that the clades with low bootstrap percentages (BS, < 50%) collapsed to polytomy. Thus, BS was plotted on the Bayesian tree (Fig. 3). Only clades supported by PP > 0.95 and/or BS > 70% were discussed below.

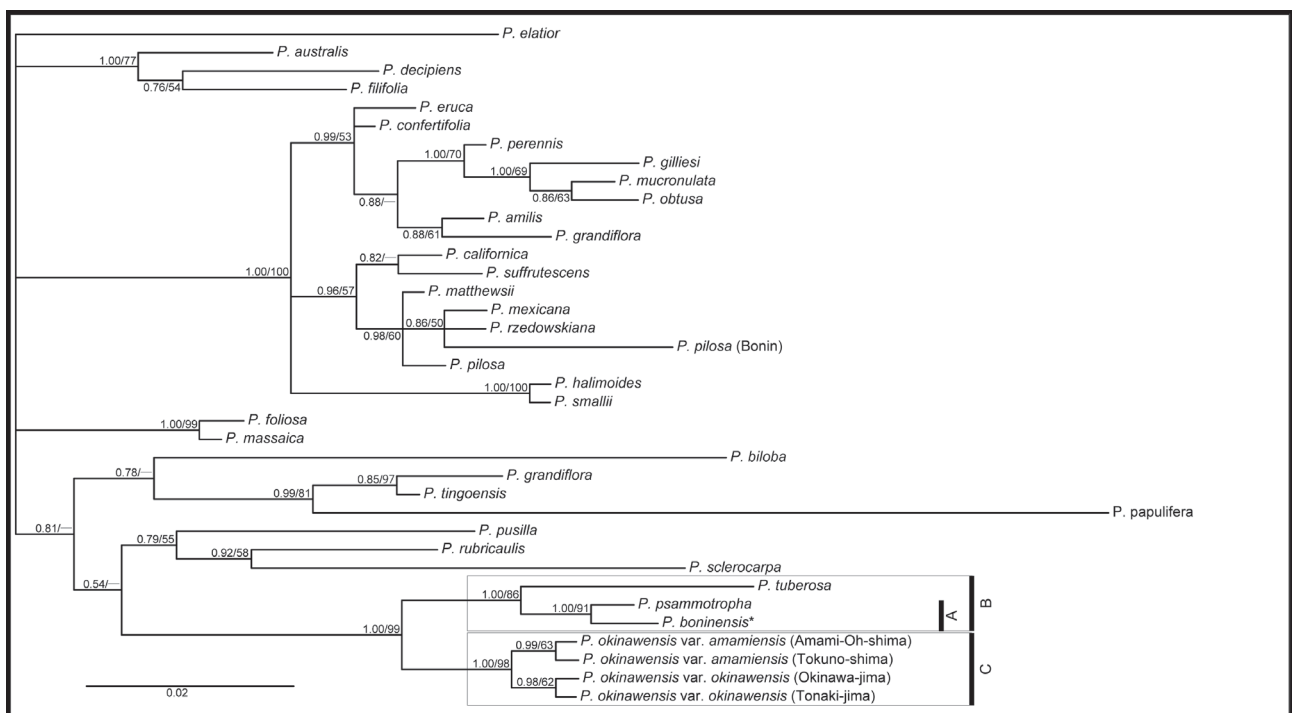


FIGURE 3. Bayesian phylogenetic tree of 38OTUs of *Portulaca* based on ITS sequences. Bayesian posterior probabilities (*left*) and bootstrap percentages in the MP analysis (*right*) are shown. **Portulaca boninensis* followed Tuyama (1983).

Both the Bayesian and MP analyses (Fig. 3) demonstrated that the plants treated as *P. boninensis* formed a well-supported clade with *P. psammotropha* (PP / BS = 1.00/91%; Clade A). Clade A was connected with *P. tuberosa* Roxburgh (1814: 91) (1.00/81%; Clade B) and Clade B was sister to a clade composed of two varieties of *P. okinawensis* (1.00/98%; Clade C). The plant identified as *P. pilosa* from the Bonin Islands and *P. pilosa* sample naturalized in USA fell in a different clade with three other congeners (*P. matthewsii*, *P. mexicana*, and *P. rzedowskiana*; 0.98/60%), being distantly related to *P. boninensis*.

Discussion

Taxonomic reconsideration of *Portulaca boninensis*

Our molecular results demonstrated that plants identified as *P. boninensis* were clearly separated from *P. pilosa*, so contrasting the hypothesis that the two species may be conspecific agreeing with Kato & Ohba (2014). The morphological observations showed that *P. boninensis* and *P. pilosa* were clearly different on the basis of the colour of the petals. Geesink (1969) noted that petal color of *P. pilosa* varied from pink to yellow, but recent taxonomic studies separated plants with yellow petal as *P. tuberosa* (see e.g., Fujita *et al.* 2008).

The present analyses revealed that *P. boninensis* is very closely related to *P. psammotropha*, which is distributed in Hainan Island of China, the southern part of Taiwan including Lanyu and Hsiao-liuqie islands, and Batan Islands of the Philippines (Chung *et al.* 2008). The two species share axillary hairs (Chung *et al.* 2008), that are absent in *P. okinawensis* (Walker & Tawada 1951, Kokubugata *et al.* 2013). Tuyama (1939) characterized *P. boninensis* by its thick and carnose roots, ellipsoidal or depressed-ellipsoidal leaves, yellow petals, four-lobed stigma, and about nine stamens. These morphological characteristics were consistent with those of *P. psammotropha* (Chung *et al.* 2008). All things stated, *P. boninensis*, described as endemic to the Bonin Island (Tuyama 1939), should be treated as a synonym of *P. psammotropha*. In the phylogeny, *P. psammotropha* was most closely related to *P. tuberosa*, treated as a race of *P. pilosa* by Geesink (1969), with yellow petals and axially hairs, but the latter was clearly distinguishable from the former in having sparse hairs surrounding the flower (Puy *et al.* 1993). In addition, the former has oblong to obovate-oblong leaf shapes (Chung *et al.* 2008), while the later has elliptic to oblong leaf shapes (Puy *et al.* 1993).

Taxonomic treatment

Portulaca psammotropha Hance (1851: 660).

Lectotype (designated here):—TAIWAN. Pratas Island, April 1831, *Wilford s.n.* (K!, image of the lectotype available at <http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000340154>).

= *Portulaca quadrifida* L. var. *formosana* Hayata (1911: 37) ≡ *Portulaca formosana* (Hayata) Hayata (1917: 7).

Type:—TAIWAN. Lanyu Island, 26 November 1899, *Miyake s.n.* (holotype TAI!, image available at <http://tai2.ntu.edu.tw/specimen/specimen.php?taiid=T00380>).

= *Portulaca insularis* Hosokawa (1932: 229).

Type:—TAIWAN. Liuchiuyu Island, 01 August 1930, *Hosokawa 1628*. (holotype TAI!, image available at <http://tai2.ntu.edu.tw/specimen/specimen.php?taiid=118821>).

= *Portulaca boninensis* Tuyama (1939: 6), *syn. nov.*

Type:—JAPAN. Bonin, Chichijima Island, 24 July 1905, *Hattori s.n.* (holotype TI!)

= *Portulaca hainanensis* Chun & How (1958: 8–9).

Type:—CHINA. Hainan, June 1932, *How 70899* (holotype SYS!).

Description:—Perennial herbs, 3–10 cm tall. Stems not articulated, diffuse, branched basally, ca. 1 mm thick, basal stems woody, prostrate, upper stems herbaceous, upright, green. Root fleshy, much branched. Leaves spirally arranged or alternate, subsessile, with axillary hairs; leaf blade 2–3 mm thick, oblong to obovate, 5–8 mm long, base obtuse, apex obtuse or rounded. Flowers solitary, about 10–15 mm in diameter. Sepals 2, ovate-deltate, about 2 mm long. Petals 5, obovate to narrowly obovate, lemon-colored, mostly without margins overlapping. Stamens 8–30. Ovary ovoid. Stigma usually trilobed or tetralobed rarely pentalobed; capsule glossy, 2–4 mm long, 2–3 mm wide.

Distribution:—North Philippine (Batan Islands), South China Sea (Hainan, Pratas Island), South Taiwan (Kenting, Liuciou, Lutao, Lanyu and the Penghu Islets), and Bonin (Ogasawara) Islands.

Ecology:—On coastal rocky slopes and sandy beaches, xeric, saline, and exposed to direct sunlight.

Typification:—Hance (1851: 660) provided a detailed diagnosis, the provenance (“*Habitat in insula corallina „Prata Island“...*”), and habitat (“*ad littora maris arenosas*”). There are two specimen at K (codes 000340153, and 000340154) collected by C. Wilford in “*Prata Island*” (= Pratas Island) as reported in the label. However, the specimen code 000340153 (image available at <http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000340153>) bears a plant collected in April 1858. As a consequence, it can be considered a post-1851 addition to the collection, it is not part of the original material for the name *Portulaca psammotropha*, and cannot be eligible as lectotype (arts. 9.2, and 9.3 of ICN, McNeill *et al.* 2012). On the contrary, the other specimen bears a plant collected in 1831 so being useful for the lectotypification purposes. The morphological characteristics of the exsiccatum matches the Hance’s diagnosis, and it is here designated as the lectotype of the name *Portulaca psammotropha*.

Morphological notes:—A diagnostic key to *Portulaca psammotropha*, *P. tuberosa*, and *P. okinawensis* is presented below:

- | | | |
|----|---|------------------------|
| 1. | Flowers surrounded by sparse hairs | <i>P. tuberosa</i> |
| - | Flowers without surrounded hairs | 2 |
| 2. | Axillary hairs present | <i>P. psammotropha</i> |
| - | Axillary hairs absent | 3 |
| 3. | Number of stamens more than 20; petals orange-yellow, obovate to narrowly obovate; stems reddish green..... | |

-*P. okinawensis* var. *okinawensis*
 - Number of stamens less than 15; petals lemon-coloured, obovate to oblanceolate; stems bright green
 *P. okinawensis* var. *amamiensis*

Biogeography notes:—Although the different climates, the floras of Bonin Islands (subtropics climate), and Ryukyus (subtropical climate) are considered similar from the phylogenetic point of view (Hara 1959, Yamazaki 1970). Therefore, it was unexpected that the species identified was not the Ryukyu endemic *P. okinawensis*, but *P. psammotropha*, that is commonly found in the Bonin Islands. The Bonin Islands represent the northwestern limit of the distribution area of *P. psammotropha* which was geographically isolated from Hainan Island of China, Taiwan, and the Philippines (Chung *et al.* 2008). Since *Portulaca* is a tropical genus and *P. tuberosa* (the sister species of *P. psammotropha*) is distributed in the Malesian and Pacific regions (Puy *et al.* 1993), we can hypothesized that the Bonin populations of *P. psammotropha* were derived from ancestral southern populations from Taiwan or the Philippines.

The Bonin Islands were formed during the Paleocene (66 Ma to 56 Ma) and rose above sea level before the middle Pleistocene, about 0.8 Ma to 0.1 Ma (Kaizuka 1977, Imaizumi & Tamura 1984). They are oceanic islands that have never been land-connected with other islands/landmasses. So, *P. psammotropha* must have migrated to the Bonin Islands oversea. The species has dry dehiscent fruits and its seeds are 0.6–0.7 mm in diameter (Tuyama 1939, Chung *et al.* 2008). Given that seeds in *Portulaca* do not float, Geesink (1969) suggested three possible mechanisms for oversea dispersal: 1) attachment to drifting wood, 2) exo- and/or endozoochory, or 3) artificial dispersal. Artificial dispersal is unlikely because Minami-iwo-jima has not been inhabited and few naturalized plants have been found in the island (Kato *et al.* 2008). On the other hand, seeds of some *Portulaca* species remained viable after floating in seawater for a few weeks (Ridley 1930). *P. psammotropha* occurs in coastal rocky slopes in Taiwan (Chung *et al.* 2008), and the Bonin Islands (Fujita *et al.* 2008, G. Kokubugata, pers. observ.). Therefore, we consider sea-current dispersal a likely scenario to explain the occurrence of the species in the Bonin Islands. The Kuroshio Current washes Taiwan and the northern Philippines, flowing northward along the Ryukyu and Japanese archipelagos and periodically meanders from Japan proper toward the Bonin Islands. The Kuroshio Current is a likely medium, although the absence of the species in the Ryukyus needs to be explained by assuming extinction or haphazard results. Long dispersal with exo- and/or endozoochory by migratory birds is also possible. Further studies, especially phylogeographic/population genetic studies, on plants showing similar distribution patterns could be used to explore the migratory origin and route of Bonin populations.

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